this arthritis model inflammation was increased in TLR2$^{-/-}$ mice. In contrast, inflammation in TLR4$^{-/-}$ mice was decreased in the arthritis model. However, in experimental OA in all strains, no obvious differences in inflammation were found, so mechanisms of action of TLR deficiencies in OA are probably unrelated to inflammation.

Conclusions: In contrast to our expectations, these studies indicate that both in TLR2 and TLR4 deficient mice OA pathology is more severe when experimental OA is induced. This suggest protective roles for both receptors in OA, in stead of the inducing roles that were anticipated. The mechanisms via which TLR2 and 4 exert this effect remain unclear, although stimulation of TLR2 and 4 by endogenous ligands such as biglycan has been shown to induce IL-10, TGFβ and HGF, which are protective mediators for OA. The differences in preference for the joint compartment may indicate different mechanisms, or differences in TLR2 and 4 distribution in the cartilage, but may also reflect strain related differences. Although further research is needed, these results indicate that during OA certain TLR pathways protect cartilage from developing OA which may lead to clues for future therapy.

A9

NOVEL FUNCTIONS OF THE TIA-1 ARTHRITIS SUPPRESSOR PROTEIN IN REGULATING TYPE II PROCOLLAGEN GENE EXPRESSION

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Purpose: One of the most important alternative splicing events identified in connective tissue biology is the developmentally-regulated processing of COL2A1. Here, exon 2, encoding a cysteine-rich domain in the N-propeptide, is included in or excluded from mature mRNA producing type IIA (+ exon 2 in chondroprogenitor cells) or type IIB procollagen (-exon 2 in differentiated chondrocytes) (Fig.1A). We recently identified a novel cis element in intron 2 of COL2A1 (Fig.1B) and proceeded to investigate what trans-acting factors bind to this U-rich region. We chose the arthritis suppressor RNA binding protein, TIA-1 (T-cell intracellular antigen 1), as a potential candidate as it has been shown to regulate alternative splicing of other genes by interacting with U-rich intronic sequences.

Methods: A COL2A1 mini-gene (Fig.1A) was co-transfected with a TIA-1-encoding cDNA construct into different chondrocyte and non-chondrocyte cells. RT-PCR of harvested mRNA was done using primers specific for the COL2A1 mini-gene (Fig. 1A). IIA and IIB cDNA isoforms were analyzed by phosphor-imaging. TIA-1 was co-transfected with COL2A1 mini-genes containing mutations in the T/U-rich region. Endogenous TIA-1 mRNA or protein was analyzed by RT-PCR or Western blotting of nuclear extracts. A GST/TIA-1 fusion protein was synthesized and used in binding assays with radio-labelled RNA or DNA probes corresponding to the T/U-rich 38 nucleotide cis element (Fig.1B). Electromobility shift or UV cross-linking assays were done to detect protein/probe complexes in the presence or absence of various competitors. Chromatin or RNA immunoprecipitation assays were done to confirm in vivo interaction of genomic DNA or pre-mRNA with endogenous TIA-1 protein in the nucleus.

Results: TIA-1 regulated alternative splicing by increasing the IIA mRNA isoform (+exon 2) in human chondrocytes and non-chondrocytes. These cells also contained the highest levels of endogenous TIA-1 compared to other cell types analyzed. Co-transfection of TIA-1 with mutant COL2A1 mini-genes suggested that the U-rich stretch (AUUUAUUUAUUU) was a potential TIA-1 RNA binding site. This was confirmed by RNA/protein binding assays. GST/TIA-1 fusion protein interacted with the wild-type RNA probe, but not with a mutant probe containing nucleotide substitutions in the U-rich region. Interestingly, TIA-1 was found to bind to the equivalent DNA T-rich probe, with a preference for single-stranded rather than double-stranded DNA. The DNA probe bound to TIA-1 with higher affinity than RNA yet, in both cases, the major TIA-1 binding site was in the T/U-rich region. Immunoprecipitation assays confirmed in vivo binding of endogenous TIA-1 to COL2A1 genomic DNA and pre-mRNA.

Conclusions: This is the first report indicating TIA-1 as a regulator of COL2A1 alternative splicing. We also report a novel function for TIA-1 by its ability to bind genomic DNA as well as RNA. We suggest TIA-1 has a dual role in regulating COL2A1 expression by functioning at the level of transcription *and* pre-mRNA splicing. TIA-1 is known to down-regulate expression of the inflammatory mediators TNF-α and COX-2 by binding adenylate/uridylate rich elements (AREs) in the 3' untranslated region of mRNA. These AREs contain repeats of the AUUUA pentameric sequence also found in the COL2A1 cis element described here. Since expression of the embryonic type IIA isoform has been detected in osteoarthritic cartilage, TIA-1 may function in promoting a tissue repair response by altering expression of specific cartilage matrix protein isoforms in addition to repressing inflammatory cytokine expression.

A10

F-SPONDIN, A NEURONAL DIFFERENTIATION FACTOR, IS UPREGULATED IN OSTEOARTHRITIS: EVIDENCE FOR REGULATION OF CHONDROCYTE METABOLISM VIA LATENT TGF-B1 ACTIVATION

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Purpose: F-spondin, first identified as a novel protein secreted by neuronal cells, is a member of a family of proteins that collectively belong to a subgroup of TSR (thrombospondin) type I class molecules. We have discovered, using Affymetrix microarray screening followed by confirmatory immunoblot and immunohistochemistry, that F- spondin expression is significantly increased in osteoarthritic cartilage. We here report studies designed to elucidate its role.

Methods: Gene expression studies of cartilage from normal and OA patients were performed using Affymetrix U95Av2 microarray. The microarray data was normalized and analyzed using dchip program. The differentially expressed gene was confirmed using TaqMan Real Time PCR. Chondrocytes were isolated from OA patients undergoing joint replacement surgery, using collagenase digestion and cultured in monolayer. Prostaglandin E2 (PGE2), was estimated using radioimmunoassay. The active and total TGF-b1 was estimated using R&D systems ELISA kits.

Results: We studied the expression of F-spondin in 18 OA and 8 age-matched normal cartilage and synovium using Affymetrix microarray. The OA specimens were obtained at the time of joint replacement surgery and the normal tissues from accident victims. F-spondin was overexpressed in OA chondrocytes (2-5 fold). The differential expression of F-spondin in cartilage was confirmed by real time PCR and western blot. The expression of F-spondin in OA cartilage was also confirmed by immunostaining. F-spondin mRNA expression was also increased in rat meniscectomy model of OA and expressed in hypertrophic and calcified zones of chick embryonic growth plates. Unstimulated chondrocytes constitutively expressed F-spondin, which decreased following exposure to IL-1 (10 ng/ml). In contrast, PGE2 (1-10 uM), significant increased F-spondin expression from 4-48h following stimulation. This effect could be mimicked by the cAMP analog, dibutyryl cAMP. Addition of F-spondin increased PGE2 production in chondrocytes, which could be inhibited by avb3 integrin blocking antibody LM609. Addition of growth factors FGF-basic, FGF-18 and TGF-b, enhanced the expression